II. REMARKS

Status of the claims

Claims 1-30, 34-51, 53-56, 69, and 70 are pending in this case. Claims 1-30, 34-51, 53-56, 69, and 70 have been examined and are rejected. By virtue of this amendment, claims 1, 10, 14, 22, 26-28, 43, and 47 are amended. For the Examiner's convenience, the pending claims, as amended, are provided in an appendix attached herewith.

In the previous Office Action, dated February 21, 1997, the Examiner rejected claims 22-27 under 35 U.S.C. § 112, first paragraph; claims 41 and 51 under 35 U.S.C. § 112, second paragraph; claims 1-3 under 35 U.S.C. § 102(b); and claims 1-13, 29, 30, and 52-56 under 35 U.S.C. § 103. These claim rejections were not maintained in the Office Action dated December 9, 1997. Accordingly, it is assumed that these rejections were withdrawn.

Generally, the claims are amended to define the invention more specifically. The claim amendments are addressed in detail below.

The amendments to the claims are meant to clarify the invention, are supported by the specification and do not add new matter. Support for these amendments can be found throughout the specification. Entry of these amendments is thus respectfully requested.

Summary of the invention

The present invention relates to methods of positively separating cells based on products secreted by the cells, compositions comprising cells separated by the disclosed separation methods, kits for separating cells, and methods for identifying and separating cells based on secreted product.

Provisional obviousness-type double patenting rejection

Applicants note that claims 1-13, 53-56, 69 and 70 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over

claims 1-6 of copending patent application Serial No. 08/441,259. Applicants will submit a terminal disclaimer upon issuance of a Notice of Allowance in either the 08/441,259 or the present application.

Objections to the specification

The Office Action notes that the application does not contain an abstract of the disclosure, and further stated that no first page of the PCT publication was filed as part of the specification of the instant application. Although an abstract was provided on the first page of the PCT publication, Applicants have amended the specification to contain the text of the PCT Abstract.

The Examiner stated that Applicants need to update the status of U.S. patent applications disclosed on the first page of the specification. Applicants note that, in the August 20, 1997 response, the specification was amended to contain a statement that the present application is a continuation-in-part of U.S. Patent Application No. 07/965,934, filed October 21, 1992. Accordingly, the status as previously updated remains current.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 1-21, 29, 30, 34-40, 43-50, 53-56, 69, and 70 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Independent claim 1 recites a method of separating cells according to a product secreted and released by the cells. Claims 2-13 depend variously from claim 1. Independent claim 14 recites a method for labeling cells with a product secreted and released by the product. Claims 15-21 depend variously from claim 14. Claims 29 and 30 recite cells and progeny thereof, and cells, respectively, separated according to the method of claim 1. Independent claim 34 recites a

kit for use in detecting cells that secrete a desired product. Claims 35-40 and 43-50 depend variously from claim 34. Independent claim 53 recites a method for identifying cells secreting product. Claims 54-56, 69 and 70 depend variously from claim 53.

Specifically, the Examiner alleged that the specification, while being enabling for the claimed method or kit which uses high viscosity or gel-forming medium, does not reasonably provide enablement for the claimed method or kit that does not use these ingredients.

Applicants disagree. As Applicants noted previously in the August 20, 1997 response, experiments described in Example 1, the results of which are illustrated in Figure 6b of the instant specification, demonstrate that successful separation of cells on the basis of secreted products was achieved, although a high viscosity medium was not used.

The data provided in the accompanying Declaration further support the fact that cells can indeed be identified and separated in accordance with the invention on the basis of secretion product using the methods and kits of the present invention without high viscosity or gel-forming media. IL-2-secreting cells were separated from a spleen cell population by capturing secreted IL-2 on the surface of the IL-2-secreting cells, labeling the cells, and separating them using fluorescence-activated cell sorting. In an analysis of the cell-surface characteristics of the labeled cells, it was demonstrated that, from a population of CD8-depleted, antigen-stimulated spleen cells, secreted IL-2 was detected mainly on $CD4^+$ blasts, with about 40% of $CD4^+$ blasts clearly staining for secreted IL-2, as shown in Figure 1b of the Declaration. In contrast, $CD4^-$ cells displayed only background staining. The labeled cells were sorted on the basis of cell surface-captured IL-2. IL-2-labeled cells were enriched by high gradient magnetic cell separation (MACS) from about 2% of all cells to 74%, as shown in Figure 2 of the Declaration. Finally, the amount of IL-2 secreted by the unseparated cells, the enriched cell fraction, and the negative cell fraction was determined. As shown in Figure 3 of the Declaration, the concentration of IL-2 in the supernatant of enriched IL-2-positive cells was 11-fold higher than

in the culture supernatant of unseparated cells and 20-fold higher than in the supernatant of IL-2-negative cells.

These experiments were performed in accordance with the claimed invention in the absence of high viscosity medium. The results demonstrate that a high degree of enrichment of IL-2-secreting cells can be achieved using the methods of the present invention, even when the method does not include the use of high viscosity medium.

Claims 69 and 70 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The Examiner states that there is no support in the specification as originally filed for the methods of claims 69 and 70. Support for claim 69 is found in the specification at, inter alia, page 25, line 28 to page 26, line 4. Support for claim 70 is found in the specification at, inter alia, page 26, line 26 to page 27, line 2. Accordingly, the invention as recited in claims 69 and 70 is adequately described.

All of the rejections on the basis of lack of enablement have been adequately addressed in view of the disclosure in the specification, as further illustrated by the evidence provided in the accompanying Declaration. Therefore, it is respectfully requested that these rejections under 35 U.S.C, § 112, first paragraph, be withdrawn.

Rejections under 35 U.S.C. § 102(b)

Claims 14, 15, 29, and 30 are rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Köhler and Schulman (1980) *Eur. J. Immunol.* 10:467-476 (hereinafter "Köhler"). Claims 22 and 23 are rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Weissman et al. (1986) *Proc. Natl. Acad. Sci. USA* 83:1463-1466 (hereinafter "Weissman").

Independent claim 14 relates to a method to label cells with a product secreted and released by the cells. Claim 15 depends from claim 14. Claims 29 and 30 relate to cells and progeny thereof, and cells, respectively, separated according to the method of claim 1. Independent claim 22 relates to a composition of matter comprising cells capable of capturing a product secreted and released by the cells. Claim 23 depends from claim 22.

In order to support a 35 U.S.C. 102(b) rejection, the cited prior art must disclose every element of the claimed invention. Among other differences, Köhler can not be said to provide the element of "separation" of cells and thus does not anticipate the claimed invention as recited in claims 14, 15, 29, and 30. Similarly, Weissman does not disclose the claim elements of amended claim 22, and thus does not anticipate this claim, nor dependent claim 23.

Regarding the rejection based on Köhler, the Examiner interprets the results of Köhler to indicate that the cell bound product (IgM) is labeled with a label moiety (complement) prior to separation, and argues that Köhler teaches the method of claim 14. The experiments provided by Köhler do not support this conclusion. Köhler discusses the use of complement as a means, not to label cells for the purposes of detection, as in the present invention, but as a means to lyse cells as part of a negative selection procedure. Since Köhler does not teach the use of complement as a label moiety, but as a cell lysis effector (thus rendering the cells lysed and not susceptible of separation), Köhler does not anticipate claim 14.

Nevertheless, in the interest of expediting prosecution and not as an aquiescence to the Examiner's assertions, claim 14 has been amended to recite "and wherein the cells are not lysed as a result of the labeling procedure". This amendment finds support in the Examples, wherein protocols for labeling cells, the subjecting them to FACS analysis are provided. Detection by FACS of a cell surface label is possible only if the cells are not lysed as a result of the labeling procedure. For example, in Example 4, a procedure for labeling live cells with secreted cytokine is provided. In this example, mouse spleen cells were stimulated with superantigen, and, after a time, the surface of the cells was biotinylated. After washing, avidin-coupled anti-IFN-γ was

added to the cells. Secreted IFN-γ captured on the surface was detected using a second antibody to IFN-γ which was labeled with digoxigenin (DIG), then a fluorochromated anti-DIG antibody. Labeled cells were analyzed by FACS. Figure 14f of the instant specification shows that, even after a 90-minute incubation, cells were labeled with IFN-γ. Obviously, the cells were not lysed as a result of the labeling procedure, since the labeled cells could still be detected by FACS. Additional support for the fact that cells are not lysed as a result of the labeling procedure is provided in the accompanying Declaration. The results shown in Figure 3 of the Declaration clearly demonstrate that cells labeled and separated according to the methods of the present invention continue to secrete IL-2 days after the labeling procedure, and were therefore not lysed as a result of the labeling procedure.

The Examiner goes on to state that claim 29 reads on progeny of cells which secrete a desired product, and argues that the progeny of labeled cells produced by the claimed invention will not be labeled because these cells are not the original labeled parent cells and the label or capture moiety would not be found on progeny cells. The Examiner goes on to argue that the cells of claim 29 are identical to hybridomas secreting a desired product, and that Köhler teaches hybridoma cells.

Claim 29 depends from claim 1, and claim 1 has not been rejected under 35 U.S.C. § 102(b) in the Office Action dated December 9, 1997. Claim 1, as amended in response to the Office Action dated February 21, 1997, recites a method for separating cells according to a product secreted and released by the cells, generally involving labeling cells with the secreted product, wherein the labeled cells are not lysed as part of the separation procedure. Obviously, this method relates to separation of cells, not lysis and negative selection of cells as taught by Köhler. Accordingly, this claim is not anticipated by Köhler, nor are claims 29 and 30, which depend from this claim.

Regarding the Examiner's assertion that "a hybridoma cell produced by the claimed method would not differ [from] any art known hybridoma cell", Applicants respectfully point out

that, as summarized in the MPEP[1], and as supported by ample case law cited therein, product-by-process claims are allowed. Claims 29 and 30 are product-by-process claims, and furthermore depend from claim 1, which recites novel subject matter. Accordingly, claims 29 and 30 recite novel subject matter.

Regarding the rejections based on Weissman, the Examiner states that Weissman teaches cells capable of capturing a product (IL-2) secreted and released by said cells wherein the capture moiety (IL-2 receptor) is anchored to the surface of said cells through an anchoring moiety (transmembrane domain of IL-2 receptor). The art-accepted meaning of the term "coupled" indicates that the coupling is not a result of normal biosynthesis. Nevertheless, and solely in the interest of expediting prosecution, Applicants have amended claim 22 to recite the limitation "the cells are modified to contain an anchoring moiety". Support for this amendment is found in the specification, *inter alia*, on page 23, lines 12-15.

All the rejections under 35 U.S.C. § 102(b) have been adequately addressed in view of the amendments to the claims and in view of the arguments presented above. Therefore, it is respectfully requested that these rejections under 35 U.S.C. § 102(b) be withdrawn.

Rejections under 35 U.S.C. § 103

Claims 14-21 and 34-51 are rejected under 35 U.S.C. § 103 as allegedly unpatentable over Köhler in view of Hunt, Chapter 55, from *Handbook of Experimental Immunology* Vol. 2, Eds. D.M. Weir et al., Blackwell Sci. 1986 (hereinafter "Hunt"), U.S. Patent 4,676,980 (hereinafter "Segal") and prior art disclosed in the specification.

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[1] MPEP 2173.05(p): "A product-by-process claim, which is a product claim that defines the claimed product in terms of the process by which it is made, is proper. *In re Moeller*, 28 CCPA 932, 48 USPQ 542, 1941 C.D. 316; *In re Luck*, 177 USPQ 523 (CCPA 1973); *In re Steppan*, 156 USPQ 143 (CCPA 1967); and *In re Pilkington*, 162 USPQ 145 (CCPA 1969). A claim to a device, apparatus, manufacture, or composition of matter may contain a reference to the process in which it is intended to be used without being objectionable under 35 U.S.C. 112, second paragraph, so long as it is clear that the claim is directed to the product and not the process.

The fact that it is necessary for an applicant to describe his product in product-by-process terms does not prevent him from presenting claims of varying scope, *Ex parte Pantzer and Feier*, 176 USPQ 141 (Bd. App. 1972)."

Claims 14-21 are discussed above. Claims 34-51 relate to kits for use in detection of cells that secrete a desired product. The kits comprise at least one bispecific antibody that has at least one antigen recognition site for at least one cell type and at least one recognition site for the secreted product.

The Examiner states that Segal teaches that bispecific antibodies can bind a cell surface antigen and another antigen, thus bringing the antigen to the cell surface. The Examiner further states that the ingredients in the claimed kit would have been used in the method taught by Köhler. The Examiner then argues that "it would have been obvious to a routineer that TNP or any desired molecule that would have been used in the method taught by Köhler would have been connected to the surface via bispecific antibody." This is incorrect.

First, Segal does not teach the use of bispecific antibodies to capture secreted product. Segal teaches bispecific antibodies wherein one antibody combining site is specific for a cell surface molecule on a cytotoxic effector cell and a second antibody combining site is specific for a cell surface molecule on a target cell; such that the bispecific antibody brings the two cells in proximity to one another, the desired outcome being killing of the target cell by the cytotoxic effector cell. Nowhere in Segal is it suggested to use a bispecific antibody in which one antibody combining site is specific for a secreted product. Furthermore, Segal teaches a method for killing cells, not positive selection. Similarly, Köhler teaches a method for killing cells, not positive selection. From a purely logical standpoint, a method for separating cells cannot be derived from two methods for killing cells.

Secondly, the Examiner's assertion that "it would have been obvious to a routineer that TNP or any desired molecule that would have been used in the method taught by Köhler would have been connected to the surface via bispecific antibody" does not appear to have a clear factual basis. Obviousness can be established only by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either in the references themselves or in the knowledge generally

available to one of ordinary skill in the art. In re Fine, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988); and In re Jones, 958 F.2d 347, 21 USPQ2d 1941 (Fed. Cir. 1992). The Examiner has not adequately explained why one of skill in the art would combine these two references. Even if combined, the references would not teach or suggest the claimed invention. Likewise, if such a suggestion were made, and it is not, since nothing in the cited references suggests the invention, it would appear that the assertion of obviousness was made using hindsight reconstruction. However, the use of hindsight in this context is impermissible, as established by ample case law, including W.L. Gore, 721 F.2d at 1553, 220 USPQ at 312-313. The fact is that Köhler neither teaches nor suggests "detection of cells that secrete a desired product", as recited in claim 34, but teaches destruction of cells secreting a product that binds TNP. Furthermore, Köhler neither teaches nor suggests the use of a bispecific antibody to capture a secreted product, but teaches direct coupling of TNP to the cell surface.

Since neither Köhler nor Segal teaches or suggests the use of a bispecific antibody to capture a secreted product, these references, neither alone nor in combination, can not render the subject matter of claims 34-51 obvious.

Claims 14-21 are directed to methods to label cells with a product secreted and released by the cells.

The Examiner states that "The product (e.g. IgM) is labeled with a label moiety (e.g. with complement) prior to separation." The Examiner argues that "Claims 14-21 are drawn to methods of labeling cells and said method would have been used in the method of Köhler". This is incorrect.

Köhler does not teach the use of complement as a "label moiety", as the Examiner states. Köhler teaches a method of lysing cells. The methods of the present invention, as recited in claims 14-21, do not relate to lysing cells. Nevertheless, in the interest of expediting prosecution, claim 14 has been amended to recite the limitation "and wherein the cells are not

lysed as a result of the labeling procedure". Accordingly, claims 14-21 are patentable under 35 U.S.C. §103 in view of Köhler.

Claims 22-28 are directed to a composition of matter comprising cells capable of capturing product secreted by the cells.

The Examiner argues that "it would have been obvious to a routineer that the method of Köhler et al. could have been practiced using labeling of the cell surface with any molecule for which negative selection of IgM producing antibodies was desired as per the method taught by Köhler for negative selection of antiTNP IgM antibodies." This is incorrect.

Köhler teaches a method of lysing cells. Claims 22-28 are directed to a composition of matter comprising cells capable of capturing product secreted by the cells. The relevance of the Examiner's assertion that "the method of Köhler et al. could have been practiced using labeling of the cell surface with any molecule for which negative selection of IgM producing antibodies was desired as per the method taught by Köhler" is not clear, since claims 22-28 are directed, not to a method, but to a composition of matter. Without wishing to be non-responsive, Applicants can not address this rejection without a further explanation of its relevance to the claimed invention.

All the rejections on the basis of obviousness have been adequately addressed in view of the amendments to the claims and the arguments presented above. Therefore, it is respectfully requested that these rejections under 35 U.S.C § 103 be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 10, 26, 34, 41, and 42 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

The Examiner states that claims 10, 26, and 34 are indefinite in the recitation of "anchor" because "it is unclear what this means or encompasses in the context recited in the claims."

Claims 41 and 42 do not recite the term "anchor". Rejection of claims 41 and 42 in this context appears to have been in error.

Regarding claims 10 and 26, the meaning of the term "anchor" is clear from the specification. Nevertheless, and solely in the interest of expediting prosecution, claims 10 and 26 have been amended to recite "anchoring moiety". Support for this phrase is found, *inter alia*, in the bridging sentence of pages 9-10; page 10, lines 12-14; page 10, lines 22-23; and page 14, lines 23-25.

Claim 34 currently recites "anchor moiety". Accordingly, the rejection appears not to apply to this claim.

Applicants submit that all the rejections on the basis of indefiniteness have been adequately addressed in view of the amendments to the claims and the arguments presented above. Therefore, it is respectfully requested that these rejections under 35 U.S.C § 112, second paragraph, be withdrawn.

III. CONCLUSION

Applicants submit that the above discussion is fully responsive to all grounds of objection and rejection set forth in the Office Action. In view of the comments above, Applicants respectfully request that all outstanding rejections be withdrawn, and that the pending claims, as amended, be allowed.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at (650) 813-5776.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this

document to **Deposit Account No. 03-1952** referencing docket no. 21230-20003.20. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

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Respectfully submitted,

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